Kinky helix

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DNA in chromatin is highly folded. Is it kinked? And does it kink in other situations?

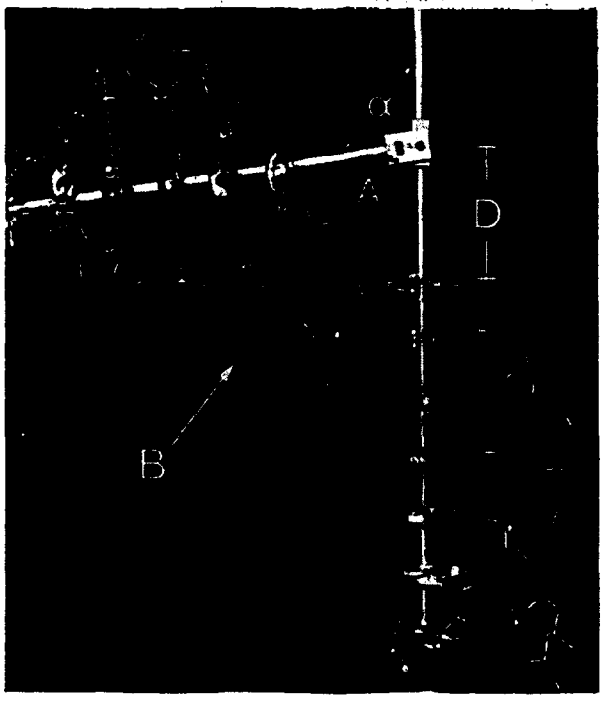
CHROMATIN is the name given to chromosomal material extracted from the nuclei of cells of higher organisms. It consists mainly of DNA and a set of small rather basic proteins called histones. Other proteins and RNA are present in lesser amounts (see for example ref. 1). Early X-ray work (for review see ref. 2) suggested that there was a structure in chromatin which repeated at intervals of about 100 Å. More recent work using nucleases[3,4] has shown that the DNA in chromatin exists in some regular fold which repeats every 200 base pairs, the best value currently being 205 ± 15 base pairs[5].

The most cogent model for chromatin has been put forward by Kornberg[6] who suggested that the basic structure consists of a string of beads each containing two each of the four major histones, each bead being associated with about 200 base pairs of DNA. Linear arrangements of beads (in a partly extended form) were first seen in the electron microscope by Olins and Olins[7] and called by them ν-bodies. The exact diameter of a bead in the wet state is rather uncertain but it is probably in the region of 100 Å. Kornberg's model suggested that DNA, when associated with histone, is folded to about one-seventh of its length. This is the value deduced by Griffith[8] from electron micrographs of the mini-chromosome of the virus SV40. A similar value has been obtained by Oudet *et al.*[9] from measurements on adenovirus 2. Other compact models have been proposed by van Holde *et al.*[10] and Baldwin *et al.*[11].

Thus the DNA in chromatin, even at this first level of structure, must be folded considerably since its length is contracted to about one-seventh. Moreover, the basic repeat of 200 base pairs (which is 680 Å long in the B form of DNA) must be folded into a fairly limited space having the dimensions of about 100 Å^3 (ref. 6).

We have found it very difficult to estimate just how much energy is required to bend DNA "smoothly" to a small radius of curvature, say 30–50 Å, bearing in mind that these numbers are not many times greater than the diameter of the DNA double helix, which is about 20 Å, and that bending a helix destroys its symmetry. We have formed the impression that the energy might be rather high. We therefore asked ourselves whether the folded DNA may consist of relatively straight stretches joined by large kinks. This paper describes a certain type of kink which can be built rather nicely and has interesting properties.

Fig. 1 General view of a model of a kink, taken from the side. For this model $d = 0$, $\alpha = 98°$, $D = 8$ Å and $\theta = 23°$ (see text). The two short lengths of backbone, connecting the two stretches of straight helix, can be seen at A. The region of van der Waals' contacts between backbones, which limit the kink angle α, is near B.



The stereochemistry of a kink

No doubt other types of kink could be built, but we have concentrated on one special type which we consider to be rather plausible. We have assumed that all the base pairs of the double helix are left intact (so that no energy is lost by unpairing them), that the straight parts of the DNA on each side of the kink remain in the normal B form, but that at the kink one base pair is completely unstacked from the adjacent one. Thus at each kink the energy of stacking of one base pair on another is lost. Naturally all bond distances and angles (including dihedral angles) have to be stereochemically acceptable.

We find that, given these assumptions, one can convincingly build a neat kink, having a large angle of kink, in one way only; or, more strictly, in a family of ways all very similar to each other. The double helix is bent towards the side of the minor groove. This can be seen in the photograph of one such model shown in Fig. 1.

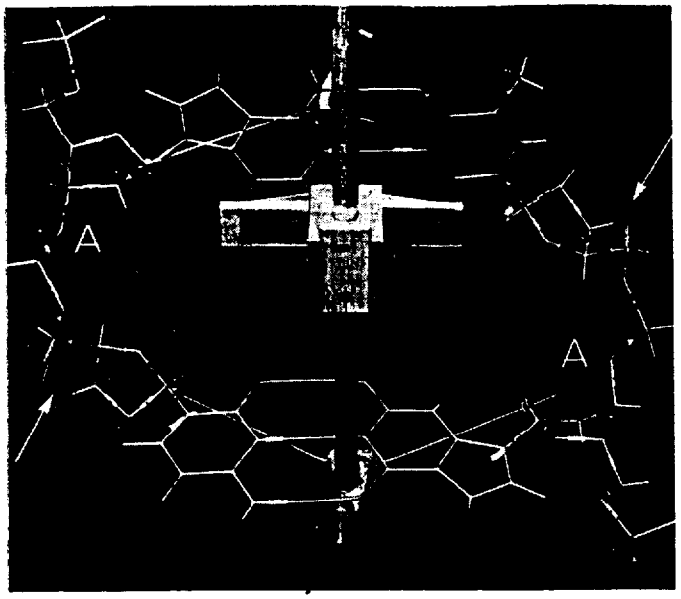


Fig. 2 View of part of the model of Fig. 1 taken approximately looking down the pseudo-dyad. It shows two base pairs, one on either side of the kink. The rest of the model has been blanked out for easier viewing. The two arrows point to the C_4'–C_5' bonds at which the chain conformation is changed by kinking—see Fig. 3. The letters A correspond to the region marked A in Fig. 1.

The structure can be built with an approximation to a dyad axis passing through the kink, though we cannot see any strong reason why such symmetry is essential in chromatin. A partial view looking along the pseudo-dyad is shown in Fig. 2.

To our surprise the configuration of the backbone at the kink can be made similar to that of the normal backbone of the B form except that the conformation at the C_4'–C_5' bond in the sugar is rotated 120° about this bond, going from one of the possible staggered configurations to another one as shown in Fig. 3. (We have arbitrarily kept the same pucker of the sugar ring as is found in the B form of DNA.)

The structure of the backbone at the kink is not one of the preferred configurations that have been listed[12,13] since these involve a weak CH · · · O close contact between the hydrogen attached to either C_6 of a pyrimidine or C_8 of a purine and the O_5' of the sugar, and by its very nature the type of kink we have assumed cannot have this at every position. In our model this contact is absent for one base of each of the base pairs immediately adjacent to the kink. As explained above, however, the backbone configuration is sufficiently close to that of the B form of DNA (except for the torsion angle about C_4'–C_5') that we feel it is acceptable stereochemically.

The nature of the base pairs on either side of the kink is immaterial to the model though presumably the energy required to unstack these base pairs will depend to some extent on their composition. We have found it difficult to estimate the free energy involved. It is probably a few kilocalories. It is obviously desirable that this figure should be determined as accurately as possible since the ease of making kinks depends on just how big it is. Another important question is how much a DNA double helix can be bent before it kinks. If we denote the mean curvature by κ (where κ equals the reciprocal of the radius of curvature) then we would expect the energy of deformation of a uniformly bent helix, per unit length, to increase at least as fast as κ^2. For a kinked helix, on the other hand, this energy increases only as κ. In this case κ is the mean 'curvature' of the segmented double helix which is proportional to the number of kinks per unit length. Thus, as is intuitively obvious, at small κ the double helix will bend, while at large κ it will kink. The value we should like to know is the radius of curvature at which it changes from bending to kinking.

There is probably an appreciable activation energy to the process of making a kink since the C_4'–C_5' bond must pass through the eclipsed configuration. For this reason we consider kinks of this type with a kink angle of about half the full 100° to be unlikely.

Common features of the family

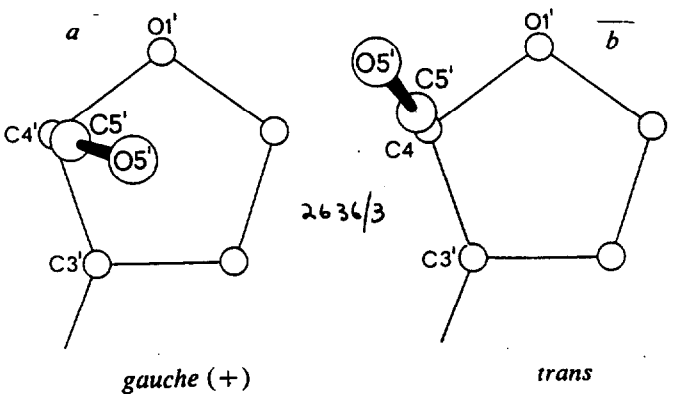
A number of very similar structures can be built along these lines and it is not obvious which is to be preferred. They all have certain features in common.

(1) The axes of the two straight parts of the DNA do not necessarily intersect exactly, but may be separated by only a small distance, d, typically about 1 Å or less. Note that d has a sign. (2) The angle between the two axes, α (projected, if necessary, on to a plane perpendicular to the line joining their points of nearest approach) is easily made more than 90° but approaches 100° with difficulty. The model shown in Fig. 1 has α=98°. At the maximum angle (for any particular model) the backbone of one straight part starts to touch the backbone of the other chain of the other straight part. This contact, marked B in Fig. 1 has a (local) dyad axis. (3) If we define the kink point as the point where the local helix axes, on either side of the kink, intersect (or, if they do not intersect, then the midpoint of the shortest straight line between the axes) then the distance, D, from the kink point to the plane of the nearest base pair is appreciable and typically in the region of 7–8 Å.

To introduce our fourth point we must first consider the relationship between three successive straight portions; that is, two kinks in succession. We assume that every kink is exactly the same. The structure formed will depend on the precise number of base pairs between the kinks. (It should be remembered that the B form of DNA has an exact repeat after ten pairs.) For example, if there are ten (or a multiple of ten) base pairs between two kinks, the structure will bend round into, very roughly, three sides of a square. If there are five base pairs between kinks (or an odd multiple of five) then the structure will approximate to a zigzag. In short the dihedral angle for three successive straight portions will depend on the exact number of base pairs between the two adjacent kinks.

We can now state our fourth point. (4) The kink imparts a small negative twist to the DNA. This is most easily grasped by imagining that the kink is made in two steps; first, the two base pairs to be unstacked are unstacked in the axial direction without kinking the backbone—this

Fig. 3 Diagrams of the deoxyribose ring showing the approximate conformation at the C_4'–C_5' bond (*a*) in the normal straight B form of DNA, and (*b*) in the proposed kink; the two sugars affected are marked in Fig. 2.



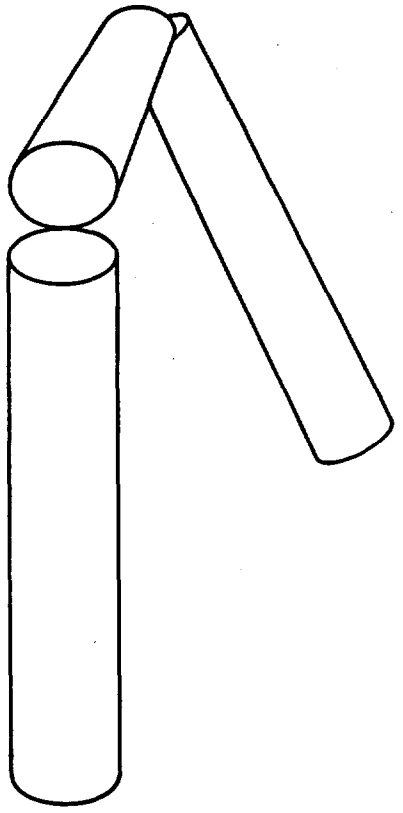


Fig. 4 Each cylinder represents a length of straight double helix. When there are ten base pairs (or an integer multiple of ten base pairs) in the middle stretch the kink has a left-handed configuration, as shown. The dihedral angle used in this paper is zero for the *cis* conformation. Its sign agrees with the usual convention that positive values less than 180° correspond to a right-handed configuration.

reduces the twist of 36° between these residues to about 10–20°—and second, that this extended structure is then kinked. The result is that if successive kinks are made at intervals of $10n$ base pairs (where n is an integer) the DNA instead of folding back to form a 'circle' follows instead a left-handed helix, though naturally a kinked helix made of straight segments (see Fig. 4).

The exact dihedral angle associated with three successive straight stretches depends somewhat on the precise details of the kink but is typically about $(m.36° - \theta)$ where m is the number of base pairs between the two kinks and θ, the dihedral correction angle, is not far from 15–20°. A very small rotational deformation of the straight portions could, however, alter this figure a little so the exact value in chromatin (if it does indeed consist of kinked helices) will probably be imposed by the histones.

(5) For any model there is a smallest number of base pairs between two adjacent kinks. For models of this family this number is usually three. In particular, the model illustrated in Fig. 1, for which $\alpha = 98°$, can be built with three base pairs between two kinks but not with only two base pairs there. This is probably true for all models of this type for which α is greater than 90°. A model with three base pairs between two kinks exposes these base pairs rather effectively.

It is easy to see that six parameters are needed to describe the relationship between any two (equal) stretches of straight double helix. If these stretches are related by a dyad axis through the kink point then only four parameters are required. These can conveniently be taken to be the four used above: d, α, D and θ.

Another family of models can be made with the kink on the side of the major groove, but such structures have a smaller angle of kink and seem rather awkward to build. We have not explored them further. A rather different type of kink, in which a base pair is undone, has been suggested by Gourévitch *et al.*[14].

The occurrence of kinks

The idea that the fold of DNA in chromatin was based on a unit of 10 base pairs was originally suggested to us by experimental evidence discovered by our colleague, Dr Markus Noll. Noll[15] has shown that the digestion of native chromatin with the nuclease DNase I produces nicks in the DNA which tend to be spaced multiples of 10 bases apart. This suggests that the DNA is folded in a highly regular way and is probably mainly on the outside of the structure[6,15]. More recent work by Noll and Kornberg (unpublished) using micrococcal nuclease points to a structural repeat at intervals of 20 base pairs. Thus a rather neat model for the most compact (wet) form of chromatin can be made in which the DNA is kinked through about 95–100° every 20 base pairs, giving a shallow kinked helix having 10 straight stretches of DNA in each 100 Å repeat. The middle stretches of this repeat would be largely protected by the histones of the bead, the flanking stretches less so. Whether this very simple model is basically correct remains to be seen.

Obviously we should ask whether DNA is kinked in other situations. One interesting possibility is that when the *lac* repressor binds to the operator site on the DNA the double helix becomes kinked. It has been shown by Wang, Barkley and Bourgeois[16] that this binding unwinds the helix by a small angle, either about 40°, or, more likely, about 90° (the value depending on the amount of unwinding assumed to be produced by the standard agent, ethidium bromide). As they point out, this is too small to allow the formation of a Gierer-type loop[17]. It is, however, just what one would expect from a small number of kinks since each kink of the kind we have described unwinds the double helix by about 15° to 25°. For example, an attractive zig-zag model can be imagined with four kinks, each spaced about five base pairs apart. This model places the two sequences related by a dyad, each of six consecutive base pairs, on either side of the first and last kinks (see Fig. 5). In this position, being near a kink, they are more exposed than they would be in a stretch of unkinked DNA.

In essence, kinking may be a way of partly exposing a small group of base pairs without too great an expenditure of energy. The exposed side of each of these base pairs is that normally in the major groove. The kink has the effect of displacing one of the phosphate–sugar backbones which normally make up the two sides of this groove. The specific pattern of hydrogen bonding sites in the major groove is thus made more accessible for a few base pairs on either side of a kink. A kink may therefore turn out to be a preferred configuration of DNA when it is interacting specifically with a protein.

Kinks may be suspected in all cases where double-stranded DNA has been shown to adopt a more compact

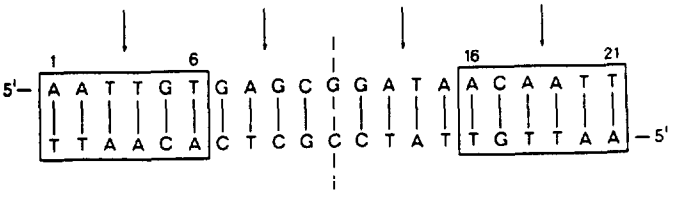


Fig. 5 The minimal base sequence of the *lac* operon, taken from Gilbert and Maxam[18]. The dotted line marks the pseudo-dyad in the base sequence. The two sets of consecutive base pairs, related by the dyad, are boxed, The arrows show one choice of positions where kinks might occur.

state than the normal double helix. Obvious examples are the folded chromosomes of *Escherichia coli*[19,20] (and no doubt other prokaryotes), the folded DNA in viruses, the ψ phase of naked DNA discovered by Lerman[21] and the shortened form of DNA in alcoholic solutions as described by Lang[22].

One should also ask whether kinks occur spontaneously, as a result of thermal motion, in double-stranded DNA in solution. The frequency at which this occurs clearly depends on the free energy difference involved. If this were, say, about 4 kcalorie then there should be one kink in about 800 base pairs which could be appreciable. Such kinks would occur mainly between A–T pairs. If the free energy were as high as 6 kcalorie this would produce one kink in about every 22,000 base pairs, which would be more difficult to detect.

At the present we have no compelling evidence which shows that DNA in chromatin is kinked rather than bent nor that kinks exist in DNA in other contexts. Nevertheless our model seems to us sufficiently attractive to be worth presenting now for consideration by other workers in the field. Kinks, if they occur, have at least two possible advantages. It has always been a puzzle how to construct hierarchies of helices in a neat way, since bending an existing helix necessarily distorts its regular structure. This distortion becomes more acute as the basic helix is coiled at higher and higher levels. A kink allows such deformations to be local rather than diffuse and makes it easier to build hierarchical models which are neat stereochemically. The other advantage is that, at a kink, several base pairs may be more easily available for specific interaction with a protein. If kinks in DNA exist they will surely prove to be important.

We thank our colleagues Drs R. D. Kornberg, M. Noll and J. O. Thomas for communicating their results to us before publication. We also thank them and our other colleagues for many useful discussions on chromatin structure.

Received April 25; accepted May 6, 1975.

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